Claims

1. An immobilized metal ion affinity chromatography purification method for purification of a recombinant proteins, said method comprising:

(a) providing carboxymethylated aspartate ligand complexed with a transition metal ion in a 2^+ oxidation state, having a coordination number of 6;

(b) loading a mixture of cell lysate comprising a recombinant protein having a polyhistidine tail to bind with said ligand; and

(c) eluting said recombinant protein with a suitable elutant to obtain a purified recombinant protein.

2. The method, according to claim 1, wherein said transition metal-complexed carboxymethylated aspartate ligand forms a carboxymethylated aspartate chelating matrix which comprises said transition metal and a polymer matrix.

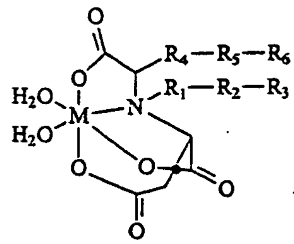
3. The method, according to claim 2, wherein said transition metal is connected to said polymer matrix by a linking arm and a functional linking group.

4. The method, according to claim 3, wherein said linking arm is selected from the group consisting of $-CH_2CH(OH)CH_2-$, $-CH_2(OH)CH_2\text{-}O\text{-}CH_2CH(OH)CH_2-$, $-(CH_2)_4NHCH_2CH(OH)CH_2-$, and $-(CH_2)_2NHCH_2CH(OH)CH_2-$.

5. The method, according to claim 3, wherein said functional linking group is selected from the group consisting of O, S, and NH.

6. The method, according to claim 2, wherein said polymer matrix is agarose.

7. The method, according to claim 2, wherein said carboxymethylated aspartate chelating matrix has the structure



wherein:

R_4-R_5-R_6 = H

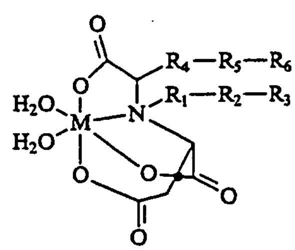
M = transition metal ion in a 2^+ oxidation state with a coordination number of 6;

R_1 = a linking arm connecting the nitrogen atom of CM-Asp with R_2;

R_2 = a functional linking group through which CM-Asp linking arm R_1 is connected to R_3; and

R_3 = a polymer matrix.

8. The method, according to claim 2, wherein said carboxymethylated aspartate chelating matrix has the structure



wherein:

R_1-R_2-R_3 = H;

M = transition metal ion in a 2^+ oxidation state with a coordination number of 6;

R_4 = a linking arm connecting the methylene carbon atom of the carboxymethyl group of CM-Asp with R_5;

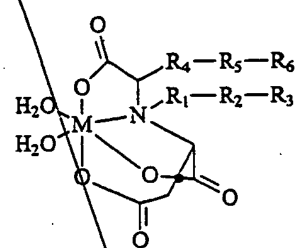
R_5 = a functional linking group through which CM-Asp linking arm R_4 is

connected to R_6; and

R_6 = a polymer matrix.

9. An immobilized metal ion affinity chromatography complex comprising a carboxymethylated aspartate ligand and a transition metal complexed thereto, wherein said transition metal ion has a 2^+ oxidation state and a coordination number of 6.

10. The complex, according to claim 9, wherein said complex has the structure:



wherein:

R_4-R_5-R_6 = H

M = transition metal ion in a 2^+ oxidation state with a coordination number of 6;

R_1 = a linking arm connecting the nitrogen atom of CM-Asp with R_2;

R_2 = a functional linking group through which CM-Asp linking arm R_1 is connected to R_3; and

R_3 = a polymer matrix

11. The method, according to claim 10, wherein said polymer matrix comprises a polymer matrix suitable for use in affinity or gel chromatography.

12. The complex, according to claim 10, wherein

M = Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+};

R_1 = $-CH_2CH(OH)CH_2-$, $-CH_2(OH)CH_2-O-CH_2CH(OH)CH_2-$, or

$-(CH_2)_2NHCH_2CH(OH)CH_2-$.

R_2 = O, S, or NH; and

R_3 = agarose or polystyrene.

13. The complex, according to claim 12, wherein

$M = Co^{2+}$;

$R_1 = CH_2CH(OH)CH_2$;

R_2 = O; and

R_3 = agarose, cross-linked or polystyrene

14. A method for synthesizing carboxymethylated aspartate agarose chelating resin, said method comprising

(a) forming oxirane-agarose;

(b) conjugating aspartic acid to oxirane-agarose; and

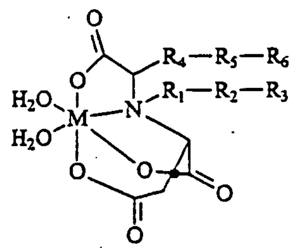
(c) washing said aspartic acid-oxirane-agarose conjugate to remove extraneously bound metals using a high ionic strength solution.

15. The method, according to claim 14, wherein said conditions for oxirane-agarose formation comprise carrying out the formation at about room temperature, overnight, adjusting to about pH 7.0.

16. The method, according to claim 14, wherein said temperature control conditions for conjugating aspartic acid to said oxirane-agarose comprise mixing at less than about 25°C, reacting at about 80°C for 4 hours, then cooling to room temperature overnight.

17. The method, according to claim 14, wherein said washing step (c) comprises use of a solution of at least 7.5% sodium hydroxide.

18. The complex according to claim 9, wherein said complex has the structure:



wherein:

R_1-R_2-R_3 = H;

M = transition metal ion in a 2^+ oxidation state with a coordination number of 6;

R_4 = a linking arm connecting the methylene carbon atom of the carboxymethyl group of CM-Asp with R_5;

R_5 = a functional linking group through which CM-Asp linking arm R_4 is connected to R_6; and

R_6 = a polymer matrix.

19. The method, according to claim 18, wherein said polymer matrix comprises a polymer matrix suitable for use in affinity or gel chromatography.

20. The complex according to claim 18, wherein

M = Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+};

R_4 = $-(CH_2)_4NHCH_2CH(OH)CH_2-$ or $-(CH_2)_4NH-$;

R_5 = O, S, NH, or CO; and

R_6 = agarose or polystyrene.

21. The complex, according to claim 20, wherein

M = Co^{2+};

R_4 = $-(CH_2)_4NHCH_2CH(OH)CH_2-$ or $-(CH_2)_4NH-$;

R_5 = O or CO; and

R_6 = agarose, cross linked, or polystyrene.

22. A method for synthesizing carboxymethylated aspartate chelating matrices, said method comprising the steps:

(a) Michael addition of the α-amino function of monoprotected α,ω-diamino acids to maleic acid;

(b) deprotecting the ω-amino functionality; and

(c) attaching the chelator primary amine molecule to a solid matrix.

23. A method for screening for protein function on a microtiter plate or filter, said method comprising the steps:

(a) immobilizing a complex of claim 1 to the plate or filter;

(b) binding said immobilized complex to the protein for which the function is being screened; and

(c) performing an assay for protein function on the bound protein.

ADD B1